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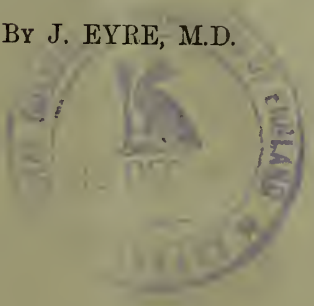
ON THE XEROSIS BACILLUS.

By J. EYRE, M.D.



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ON THE XEROSIS BACILLUS.

ON THE XEROSIS BACILLUS.[1]

By J. Eyre, M.D.

From the Bacteriological Laboratory, Guy's Hospital.

Plate IV.

Recently, whilst investigating a series of cases of follicular conjunctivitis, which differed in some respects from the ordinary form assumed by that affection, a micro-organism was isolated from the conjunctival secretion, so closely resembling the diphtheria bacillus that I at first supposed them to be identical. A careful and comparative study, however, of the two organisms, with regard to their mode of growth upon various media, together with their morphological appearances, convinced me of my error; and it seemed to me, in view of the importance to ophthalmic surgery of lesions associated with, and dependent upon, the Klebs-Löffler bacillus, that an account of the results obtained might be of some interest.

Before proceeding to describe the bacillus—which I now know as the xerosis bacillus of Neisser—a few words are necessary as to the sources from which it was obtained.

Cases.

The form of conjunctivitis in which the organism occurred was characterised clinically by a number of small, irregularly oval-shaped, pinkish, œdematous bodies (resembling somewhat the flabby granulations covering the base of a varicose ulcer), situated in the lower conjunctival fornix, and not encroaching upon the ocular conjunctiva. These were probably due to hypertrophy of the papillæ and subendothelial adenoid tissue. Injection of the conjunctival vessels, lachrymation, photophobia, inability to continue near work, distress at night and when using artificial light, were among the symptoms.

Twelve such cases were examined, 6 males and 6 females. The micro-organism was isolated from each case. Of the females, 2

[1] Communicated to the Pathological Society of London, 17th December 1895, and published with permission of the Council.

were classmates at school; the remaining 4 were members of the same family—an interval of about a week being noted between the onset of the attack in the mother and in the three children. Among the males no such connection could be traced.

[In a thirteenth case—one of acute trachoma—a sago-granule was opened, with aseptic precautions, and the fluid taken from its interior was found to contain this organism in pure culture.]

With two exceptions the organism was obtained from the conjunctival secretion in pure culture. One of these exceptions was a brewer's lad, whose secretion contained also a white torula. The other exception was one of the schoolgirls, the *Staphylococcus pyogenes aureus* being associated with the xerosis bacillus.

I have also, up to the present time, examined the conjunctival secretion taken from 25 pairs of normal eyes, by means of coverslip preparations and cultivations, but have not succeeded in detecting this bacillus.

History.

The xerosis bacillus was discovered in large quantities by Kuschbert and Neisser, in a condition known as xerosis conjunctivæ, and their observations have been confirmed by many authors since.

Babes found it in 8 cases of trachoma, whilst Deyle isolated it from 15 cases of chalazion formation, and indeed considered that it was the cause of the disease, as he was able to reproduce such formations in animals by means of inoculations of this organism.

It has been isolated also from other situations. For instance, Neisser states that he found it in cases of soft chancre, vaginal discharge, ulcers of the leg, etc.

There is some difference of opinion as to its occurrence in the normal conjunctival secretion. On the one hand, Fränkel and Uthoft state that it is frequently present in normal eyes. On the other hand, Franker examined 120 normal conjunctivæ without being able to discover the bacillus.

Biology and Morphology of the Xerosis Bacillus.

The xerosis bacillus is a non-motile, non-liquefying facultative anærobic, non-sporing bacillus.

Coverslip preparations, made directly from the conjunctival secretion, showed a straight or very slightly curved bacillus about 1·75 μ in length by ·5 μ broad, staining irregularly, some portions of the protoplasm, especially at the poles, taking the stain (carbol-methylene-blue) deeply, whilst others were almost colourless, thus giving rise to a beaded appearance.

First cultivations were obtained by streaking the conjunctival

secretion upon inspissated blood serum, as it was found, by means of simultaneous plantings upon other media (agar, glycerine-agar, and gelatine), that it would not grow upon them.

At the end of 24 hours no growth was visible to the naked eye, nor could any be made out microscopically. After a period varying from 36–48 hours after inoculation an abundant growth made its appearance. Subcultures from this grew readily on all ordinary media in 18–24 hours, and it is these early subcultures I now propose to describe, giving at the same time, in parallel column, a description of a fairly typical long Klebs-Löffler bacillus when cultivated under similar conditions.

DESCRIPTION OF EARLY SUB-CULTURE OF B. DIPHTHERIÆ AND XEROSIS BACILLUS.

KLEBS-LÖFFLER BACILLUS.	XEROSIS BACILLUS.
BLOOD SERUM (INSPISSATED).	
MACROSCOPIC.	
Similar growths but moist—not scaly.	Growth visible to the naked eye at the end of 12–18 hours, as small spherical opaque white colonies, having a slightly heaped-up appearance in the centre, and with clearly defined regular margins. The growth is dry and scaly-looking, adhering firmly to the surface of the medium. The water of condensation may also contain a few scaly colonies, which have been washed off the lower part of the surface.
MICROSCOPIC, *at* 12, 18, *and* 24 *hours* (see Plate IV. Fig. 1).	
The majority of the bacilli measure 1·8 μ in length and ·8 μ in breadth, and stain intensely in the centre. Forms are also present about twice this length, and staining segmentally. There are also present long bacilli, slightly curved, and clubbed at either end, and a few small pear-shaped forms.	The bacilli are generally straight, occasionally two elements being joined together, either in the same straight line, or at a slight angle. The bacilli are collected together in small groups of 6, 10, 20, and upwards. There is very little segmentation to be noticed at this stage. Another form also occurs—slightly curved bacilli, usually single or in pairs, exhibiting a fair amount of clubbing. The great majority are distinctly shorter than the Klebs-Löffler bacilli, are more regular in size, and more even in staining.
At 48 *and* 72 *hours*, 3 *and* 7 *days*.	
The greater number of the bacilli appear as darkly staining forms, short pear-shaped, clubbed forms, often transversely segmented, and long curved forms swollen at either end, and marked out with darkly staining segments. A fair proportion are unstained, or but faintly coloured. Metachromatism is common especially with the clubbed ends of the bacilli.	Resemble the Klebs-Löffler bacilli closely. The individual bacilli are clubbed and segmented, both characteristics frequently being united in one element. Short pear-shaped bodies, transversely striated, are seen. The staining is irregular in depth and character. The grouping of the bacilli into small bunches is still a marked feature; metachromatism is fairly common.

Klebs-Löffler Bacillus.	Xerosis Bacillus.
GLYCERINE SERUM (Inspissated).	
Blood serum to which about 5 per cent. of sterile glycerine has been added, previous to inspissation, forms an excellent medium.	
Macroscopic.	
The character of the growth, though rather more luxuriant, is similar to that described under the previous heading (*vide* Blood Serum).	
Microscopic.	
The formation of clubbed forms, darkly-staining oval or pear-shaped masses of varying size, and long swollen bacilli is very remarkable. Metachromatism is extreme.	Clubbed forms are noted early, and segmentation of the protoplasm shows up well, giving some of the bacilli a beaded appearance. Metachromatism is very pronounced. The arrangement of the bacilli in small groups is well marked. The morphological characters are precisely those met with in the conjunctival secretion.
AGAR-AGAR.	
Agar-agar, especially that to which 5–7 per cent. glycerine has been added, forms a very good medium.	
Macroscopic.	
Plates.—The colonies are indistinguishable from those formed by the xerosis bacillus.	*Plates.*—The superficial colonies are whitish in colour by reflected, and yellowish brown or buff, by transmitted light. The outline is irregular and ill defined, and the surface rough and granular, showing a darker and better defined central or sometimes laterally placed vegetation centre. The deeply situated colonies are roughly circular or oval in shape, the outline is fairly sharp and well defined, and the surface appears coarsely granular.
Streak.—The growth is similar to that due to the xerosis bacillus at first, but after 48–60 hours the colonies of the Klebs-Löffler bacillus have increased markedly in size, are confluent and heaped up (may even reach a diameter of $\frac{1}{8}$ in.).	*Streak.*—The growth is easily visible to the naked eye at the end of 18–24 hours, and consists of small, discrete, spherical, whitish, semi-transparent colonies, resembling those formed by pyogenic streptococci, rather than those due to the Klebs-Löffler bacillus. In from 3–6 days the individual colonies have perhaps increased slightly in size, but never become confluent or heaped up.
Stab-cultures do not grow so well as those of the xerosis bacillus, especially in the deeper parts of the stab.	*Stab.*—In stab-culture it grows well, and after six hours appears, at the lower end of the needle track, as a chain of distinct spherical colonies. After a time the majority of the colonies coalesce, and the needle track shows up as a distinct line.
Microscopically, *at* 24 *and* 48 *hours* (see Plate IV. Fig. 2).	
Similar in morphology to bacilli grown upon blood serum. The bacilli stain more evenly, and numerous gigantic forms, second in size only to those grown on potato, are present.	The bacilli are distinctly shorter that the Klebs-Löffler bacillus. Segmented rods are fairly common, and there is a certain amount of clubbing to be noticed. The combination of clubbed and segmented forms is rare. The tendency of the bacilli to collect in small clumps is still present. At 4–6 days involution forms are common, and large numbers of unstained bacilli can be detected. Clubbed and segmented forms are common, and the only points of distinction from the Klebs-Löffler bacillus are that it is shorter, and is arranged in small groups.

Klebs-Löffler Bacillus.	Xerosis Bacillus.
GELATINE (7–10 per cent.).	
Macroscopic.	
Growth similar to that of the xerosis bacillus, but decidedly more vigorous. Colonies can be recognised in 12–24 hours.	This material, probably because it is incubated at a low temperature (18°–20° C.), does not form a good medium—no growth is visible to the naked eye under 48 hours.
	Streak.—In streaks the growth is scanty, opalescent, and nearly transparent, forming a thin dryish film, strictly limited to the needle track. Has a pearly lustre by transmitted light.
Stab.—Growth in the depths of the needle track is very scanty.	*Stab.*—In stabs, growth occurs in the depths of the needle track as small isolated spherical colonies, semi-transparent and slightly opalescent.
	The medium is not liquefied.
Microscopic, *at* 48 *hours* (see Plate IV. Fig. 3).	
The bacilli differ in size from those grown upon serum, in being distinctly shorter. The central portion stains well, and there is but little segmentation to be noted, and still less clubbing.	The bacilli are longer than those grown on any other medium. Some are straight, whilst the majority are slightly curved, and collected together in small groups. The staining, though never good, is even throughout, no segmentation or clubbing being observed.
BOUILLON.	
Macroscopic.	
When cultivated in tubes both organisms grow well, and in about 24 hours give rise to a granular opacity of the medium. After 48–60 hours the broth again becomes clear, owing to the subsidence of the bacilli to the bottom of the tube.	
Reaction in Neutral Broth.	
When this is used the reaction gradually becomes acid, and is markedly so in 24–30 hours. After this the acidity slowly diminishes, and eventually the broth becomes strongly alkaline.	For the first 48 hours no change is observed in the reaction of the medium. After this time it is found to be faintly alkaline, becoming distinctly so in about 60 hours.
Reaction in Alkaline Broth.	
When bouillon having a distinctly alkaline reaction is used, the amount of acid produced by the Klebs-Löffler bacillus is not only sufficient to reduce the reaction to neutrality, but also to render it distinctly acid.	Growth takes place rather more rapidly than in neutral broth, and after 36–48 hours the alkalinity is slightly increased.
Microscopic.	
The prevailing form is a short squat bacillus, about ·75 μ in length. A few elongated forms are seen, but these very rarely exceed 1·8 μ.	The rods are short, ·75 μ in length—the breadth apparently varying with the stain—quite straight; no clubbed forms are to be seen; they still retain their tendency to collect in small groups.

Klebs-Löffler Bacillus.	Xerosis Bacillus.
MILK.	
Macroscopic.	
The organisms grow equally well in this medium without producing coagulation. *Reaction.*—The observations upon this point, described under the heading of Bouillon, apply to the medium also.	
Microscopic.	
The morphological characters of the two organisms, grown in milk, are similar to those described under bouillon.	
POTATO.	
Macroscopic.	
Usually invisible, or is indicated as a thin dry glaze after several days. In 24 hours, at 37° C., microscopic examination shows an abundant growth.	Usually invisible, or appears as a thin dry glaze at the end of 36–48 hours. On microscopic examination growth is very scanty, and dies in a few days.
Microscopic.	
Enormous clubbed forms are present, and are of larger size and thicker than those formed on any other medium. The common segmented forms are few in number. Irregular shapes are particularly numerous, and small, coccus-like bodies are also present.	At 48 hours the bacilli are very short, and there is a marked absence of clubbing. Segmentation is never observed. The organisms are arranged in pairs, end to end, and in groups of two or three such pairs, side by side, approaching in appearance more nearly to the short variety of the diphtheria bacillus. The xerosis bacillus stains badly and slowly, and numerous unstained organisms are noticed. At 60 hours none stain deeply—the majority are unstained.

The foregoing descriptions apply more particularly to the early generations of the xerosis bacillus. After sub-cultivating on blood serum through a number of (say 10) generations, the organism becomes distinctly shorter and more slender, and more curved. The protoplasm stains evenly throughout the length of the bacillus, and neither clubbing nor segmentation is observed. Nor is the frequently mentioned characteristic "clumping" so well marked. The peculiarities which render the xerosis bacillus liable to be mistaken for the diphtheria bacillus, and which are lost when the organism is cultivated for many generations *in vitro*, can be restored by cultivating upon glycerine blood serum (inspissated) for one or two generations.

Stains.

The xerosis bacillus stains well with all the aniline dyes, and is not decolorised by Gram's method. The stains giving, perhaps, the

best results are carbol-methylene-blue and aniline gentian-violet. With the former, one frequently gets, especially in old (4–6 days) agar cultures, remarkably good examples of metachromatism—portions of the organism being stained a deep blue, whilst others present a magenta tint. With the latter stain the bacillus appears somewhat thicker than when other dyes are used. The same peculiarity may be observed in staining the Klebs-Löffler bacillus with aniline gentian-violet. This may be due to the sheath also taking up the stain.

Temperature.

Undoubtedly the optimum temperature for the growth of the xerosis bacillus is 37° C. Growth takes place up to 42° C. or 44° C.; beyond that temperature growth is inhibited. The thermal death point is reached at 58° C., an exposure to that temperature for 10–15 minutes being sufficient to kill the organism. Growth will also take place at 19° C., but is very feeble and slow. No growth whatever occurs below 15° C.

Vitality.

The length of time the organism will retain its vitality upon artificial media is very variable. The average time is about 3–5 weeks. I have succeeded in obtaining a growth when inoculating from a blood-serum culture, 4 months old. I have also failed in the case of some cultures only 3 weeks old.

Inoculation Experiments.

Guinea-pigs were inoculated with 48-hour broth-cultures of the bacillus derived from each of the 13 cases; the dose, irrespective of the weight of the animal, being 1 c.c. injected subcutaneously. At the end of the 48 hours a varying amount of œdema had appeared at the seat of inoculation. This lasted for 2 or 3 days, was then gradually absorbed, and in 10 days nothing abnormal could be detected. The animals never refused food nor seemed in any way inconvenienced by the introduction of the cultures into the subcutaneous tissue.

Animals were killed at intervals of 1, 2, and 3 months after inoculation, and beyond a slight cicatricial thickening at the seat of inoculation nothing could be found.

At varying intervals after the œdema had subsided, 48-hour broth-cultures of the Klebs-Löffler bacillus were inoculated subcutaneously. These invariably killed the animals in about 3 days, and all the characteristic post-mortem appearances due to this organism were present, viz.:—

At the site of inoculation.—Extensive local œdema, due to a fibrino-purulent exudate (and in which living Klebs-Löffler bacilli

may be demonstrated), associated with more or less hyperæmia and ecchymosis.

Serous cavities.—Excessive serous effusion in the peritoneal, pleural, and pericardial cavities.

Liver and kidneys.—These sometimes exhibit fatty degeneration in a most marked manner. (The heart muscle also shows this change, though but rarely.)

Suprarenals.—Enlarged and hæmorrhagic.

Spleen and lymphatic glands.—Swollen, reddened, and sometimes showing hæmorrhagic patches.

With regard to the possible production of a toxine by the xerosis bacillus, the following experiments were performed:—

Flasks containing 100 c.c. of neutral bouillon were inoculated with xerosis bacilli from all the cases, and incubated at 37° C. for 2 months. The broth was then filtered through a Pasteur filter, and 1 c.c. of the clear sterile fluid inoculated subcutaneously into guinea-pigs, weighing about 250 grms. On the following day some slight œdema was observed at the seat of inoculation. This cleared up in 2 days, and the animals were not further affected.

Control experiments with diphtheria toxine, prepared under the same conditions, were made with the result that ·5 c.c. killed a guinea-pig of the same weight in 3 days.

True Conjunctival Diphtheria.

As a contrast to the preceding cases, from which the xerosis bacillus was isolated, I now quote a recent case of true conjunctival diphtheria.

The patient was a boy æt. 4 years. Both eyes were affected, the lids being painful, red, and swollen, and could only be separated with difficulty owing to the brawny infiltration of the subcutaneous tissue. The ocular conjunctiva was chemosed; the palpebral portion congested and thickened, presenting patches of a pale greyish-yellow membrane, which stripped off easily, leaving a raw bleeding surface below. A thin milky white discharge, slight in quantity, was also noted. Under treatment, the symptoms rapidly subsided, but within a month of the disappearance of the membrane from the conjunctiva the child developed a well-marked and progressive paresis of the extensor muscles of the left forearm and hand.

Bacteriological Examination.

Coverslip preparations made direct from the discharge revealed the presence of numerous polynuclear leucocytes and some squamous epithelial cells, whilst the bacteria present consisted of staphylococci and slender bacilli, about 1·75 μ in length, and presenting a beaded

appearance when stained with carbol-methylene-blue. None of these bacilli were clubbed, and I was totally unable to decide from this examination whether the organism in question was the Klebs-Löffler bacillus or the xerosis bacillus.

A blood serum tube was then inoculated with some of the discharge from the lower conjunctival fornix (left eye), and incubated for 16 hours at 37° C. At the end of this time a scanty growth was observed, consisting of several small, rounded, raised opaque white colonies with sharp cut edges, averaging 1 mm. in diameter, which microscopically consisted of the form of the Klebs-Löffler bacillus known as the long variety. At the end of 24 hours colonies of staphylococci were noted, and also an irregular growth of *B. subtilis* along the edges of the nutrient surface.

Inoculation Experiment.

From the growth on blood serum, agar plates were made, and a pure culture of the *B. diphtheriæ* obtained; from this latter a broth tube was inoculated, and after 48 hours' incubation at 37° C., 1 c.c. of the resulting growth was inoculated into the subcutaneous tissue of the abdomen of a guinea-pig weighing 258 grms. At the end of 60 hours the animal was dead, and a post-mortem examination demonstrated the presence of those pathological conditions associated with death due to the *B. diphtheriæ*.

[To Dr. Washbourn my best thanks are due for his kindness in performing these inoculation experiments for me, and also for much valuable advice and assistance during the course of these experiments.]

Differential Summary.

In differentiating the xerosis bacillus from the Klebs-Löffler bacillus we are saved all trouble in the case of first cultures by the fact that the former does not grow on blood serum at 37° C. under 36–48 hours, whilst the latter makes its appearance in 18–24 hours.

At the other extreme, with cultures some 15–20 generations old, there is likewise very little difficulty in distinguishing between these two organisms, as the xerosis bacillus then appears as a much shorter, more slender and more curved bacillus, exhibiting neither segmentation nor clubbing.

But in the case of early subcultures from the first culture the circumstances are entirely altered, and we have to deal with an organism closely resembling, in its general characters and mode of growth, the Klebs-Löffler bacillus—an organism moreover which has no one single persistent peculiarity which will enable us to say definitely, this is the xerosis bacillus.

We have therefore to depend upon the sum total of the cultural

and morphological differences—minute in themselves—picked out during the course of numerous observations. These differences have been indicated in the foregoing parallel descriptions in some detail, but to sum up broadly, the chief points of distinction between the xerosis bacillus and the diphtheria bacillus are as follows:—

1. After inoculation from the secretion, upon blood serum, colonies of the xerosis bacillus do not appear under 36 hours—those of *B. diphtheriæ* appear in 16–18 hours.

2. When grown in neutral bouillon or milk the xerosis bacillus never gives rise to an acid reaction—*B. diphtheriæ* invariably does so.

3. When grown upon potato the xerosis bacillus rapidly degenerates and dies—*B. diphtheriæ* grows with more vigour and to a greater size than on any other medium.

4. When grown upon 10 per cent. gelatin, colonies of the xerosis bacillus are not visible to the naked eye under 48 hours—*B. diphtheriæ* colonies can be recognised in 12–24 hours.

5. The invariably innocuous nature of the bouillon cultures of the xerosis bacillus, when inoculated into the subcutaneous tissues of animals susceptible to the *B. diphtheriæ*.

As to the exact nature of the xerosis bacillus—whether it be a non-virulent and slightly altered species of the *B. diphtheriæ*, or a totally separate and distinct bacillus, it is impossible at present to decide.

[The expenses of the foregoing experiments were defrayed in part by a grant from the Scientific Grants Committee of the British Medical Association.]

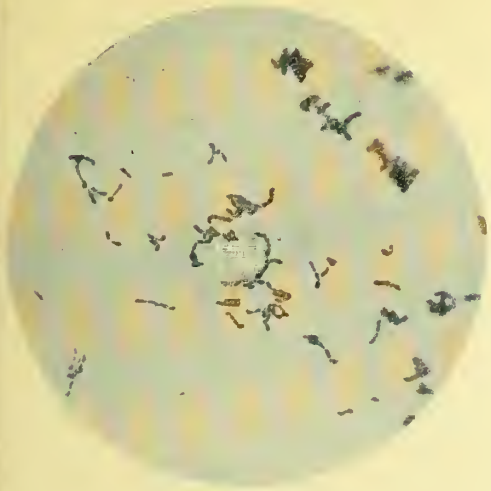

FIG. 1.

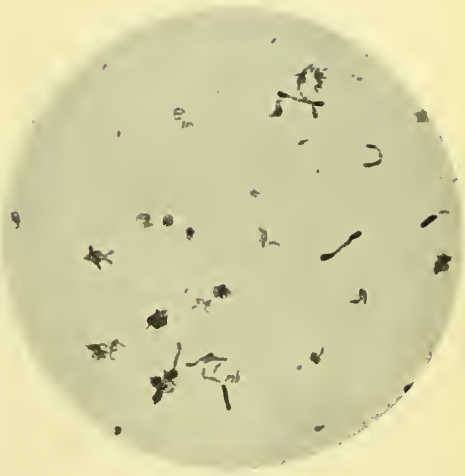

FIG. 2.

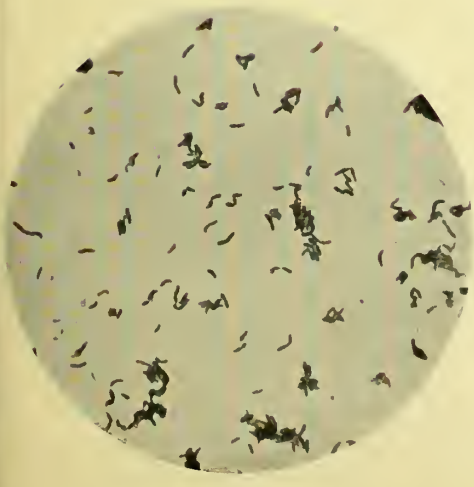

FIG 3.